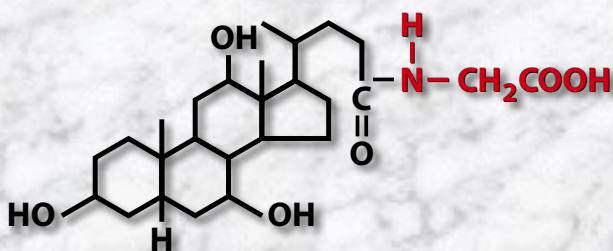


# TOTAL BILE ACIDS TEST AND CLINICAL DIAGNOSIS



**Glycocholic acid**

- **TBA & Liver Diseases**
- **TBA & HCV Treatment**
- **TBA & Pregnancy**
- **TBA & Veterinary Use**

**New**

**A Sensitive Enzyme  
Cycling Based  
Total Bile Acids Assay**

*FDA 510K approved and CE marked*



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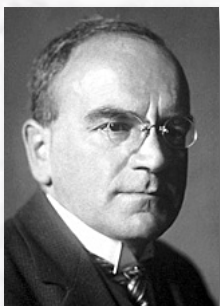
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## Introduction

Bile acids are 24-carbon steroids formed from cholesterol in the liver. Five major bile acid forms compose over 99% of the bile acid pool formed in body fluids. The chemistry and physiology of bile acids have been extensively studied, and the pioneering work on the molecular structural determination of bile acids was mainly accomplished by Dr. Heinrich Otto Wieland at the University of Munich, Germany, in 1920s.

“For his investigations of the constitution of the bile acids and related substances,” Dr. Wieland was awarded the Noble Prize in Chemistry in 1927.

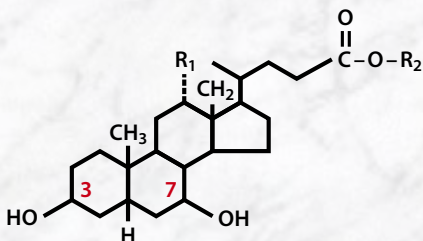
In the last half century, the chemistry and biology of bile acids have been well developed, and serum total bile acids (TBA) level as an indicator for liver diseases has been well established and accepted in clinical practices. This brochure summarizes serum TBA as a marker for clinical diagnosis of liver diseases, prognostic test for HCV, testing for cholestasis during pregnancy, TBA for veterinary testing, and the methods for serum TBA testing.



### The Nobel Prize in Chemistry 1927



Heinrich Otto Wieland



Cholic acid:  $R_1 = \text{OH}$ ,  $R_2 = \text{H}$

Chenodeoxycholic acid:  $R_1 = R_2 = \text{H}$

Glycocholic acid:  $R_1 = \text{OH}$ ,  $R_2 = \text{NH-CH}_2\text{-COOH}$

Taurocholic acid:  $R_1 = \text{OH}$ ,  $R_2 = \text{NH-CH}_2\text{-CH}_2\text{SO}_3\text{H}$

**Figure 1. Structures of Bile Acids and their Conjugates**

# Bile Acids and Compositions

The liver synthesizes two primary bile acids, cholic acid and chenodeoxycholic acid from cholesterol. The primary bile acids are converted to the secondary bile acids, deoxycholic acid and lithocholic acid by intestinal bacteria. A fraction of chenodeoxycholic acid is also transformed into the tertiary bile acid, ursodeoxycholic acid, in the liver. All bile acids secreted by the liver are conjugated with an amino acid, either with glycine or with taurine. The conjugated bile acids form further complex with sodium to become bile salts. In clinical diagnosis, TBA testing refers to the testing of the sum of all these forms of bile acid conjugates (primary, secondary, and tertiary bile acids and their conjugates). The average bile acid composition of healthy human adult bile is 38% cholate conjugates, 34% chenodeoxycholate conjugates, 28% deoxycholate conjugates, 1-2% lithocholate conjugates as shown in Table 1.

Class	Name	Chemical	Forms of Conjugates	Percent of Total Bile Acid Conjugates in Healthy Human Adults
Primary	Cholic acid Chenodeoxycholic acid	3 $\alpha$ -7 $\alpha$ , 12 $\alpha$ - Trihydroxy-5 $\beta$ cholanolic acid 3 $\alpha$ ,7 $\alpha$ -Dihydroxy-5 $\beta$ -cholanolic acid	Glycine or Taurine Glycine or Taurine	36 - 38% 32 - 34%
Secondary	Deoxycholic acid Lithocholic acid	3 $\alpha$ ,12 $\alpha$ -Dihydroxy-5 $\beta$ -cholanolic acid 3 $\alpha$ -Hydroxy-5 $\beta$ -cholanolic acid	Glycine or Taurine Glycine or Taurine	26 - 28% 1 - 2%
Tertiary	Ursodeoxycholic acid	3a,7b-Dioxycholanolic acid	Glycine or Taurine	< 1%

Table 1. Major bile acids in body fluids

## Physiological Functions of Bile Acids

Bile acids are the major constituents of bile, and in mammals, compose approximately 67% of bile secretion. Bile acids are released from the liver as conjugated salts into the small intestine via the bile duct during intestinal contraction. Because conjugated bile acids possess both polar and non-polar regions, molecules like bile acids are able to solubilize biliary lipids, act like a detergent to emulsify dietary fat droplets through the formation of mixed micelles. This significantly increases the surface area of fat, making it available for digestion by lipase, which otherwise can not access the interior of lipid droplets. Bile acids are lipid-carriers and are able to solubilize many lipids by forming mixed micelles with fatty acids, cholesterol for the solubilization and absorption of fat-soluble vitamins such as vitamin E. The ability of bile acids to solubilize cholesterol in bile is the major mechanism of cholesterol elimination from the body to prevent cholesterol accumulation with the attendant risk of atherosclerosis.

## Bile Acids Metabolism and Enterohepatic Circulation

More than 90% of the bile acids are actively reabsorbed (by a sodium-dependent co-transport process) from the ileum into the hepatic portal circulation from where they are cleared and re-secreted by the liver to once again be stored in the gallbladder. This secretion/reabsorption cycle is called the enterohepatic circulation as shown in Figure 2. The bile acids pool cycles 5-10 times daily through the enterohepatic circulation where it is almost completely confined. The liver normally clears 20 g of bile salt from the blood each day. Less than 1% of the total bile acid pool is present in the peripheral blood due to the high efficiency of the hepatic transport mechanism for bile acids.

Normally, the liver is very efficient at capturing and removing bile acids from the hepatic-portal circulation. This is why the peripheral blood levels of total bile acids are quite low in healthy subjects. The levels of circulating bile acids at any moment are determined by the balance between intestinal absorption and hepatic elimination of bile acids. However, when the

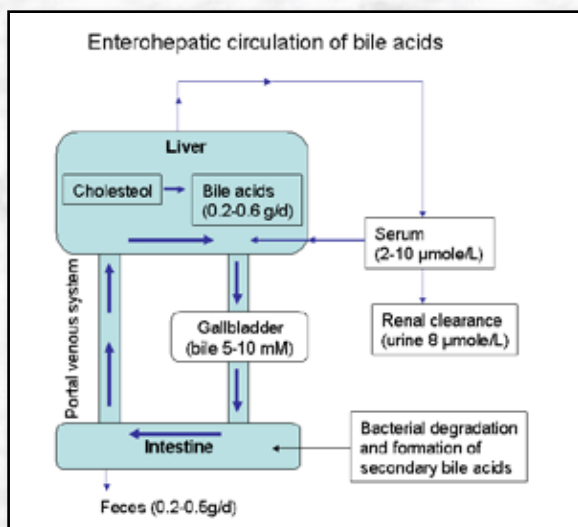


Figure 2.

enterohepatic circulation system is impaired, blood levels of bile acids are increased as a result of diminished hepatic elimination of bile acids from the portal blood, which results from diminished hepatic clearance and from portosystemic shunting as shown in Figure 3.

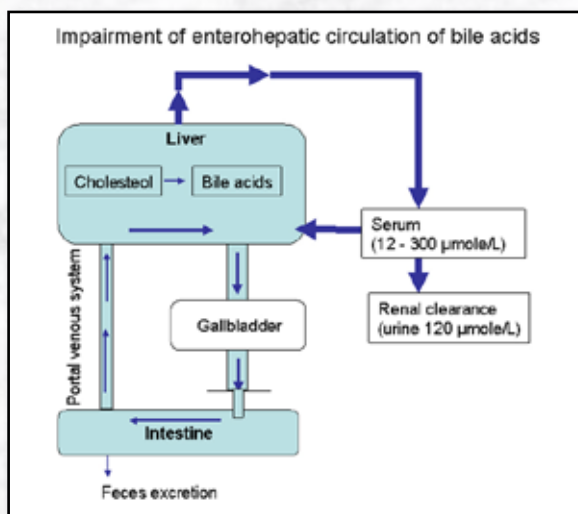


Figure 3.

## Diagnostic Value of Serum Total Bile Acids Test

The liver removes bile acids effectively from the portal circulation because of the presence of bile acid transporters on the sinusoidal membrane of hepatocytes. The high extraction efficiency (first-pass clearance is 75-90%) is the reason for low peripheral blood levels of total bile acids (2-10  $\mu\text{mole/L}$ ) compared with portal concentrations of bile acids (60-80  $\mu\text{mole/L}$ ). Any decrease in the extraction efficiency caused by a decrease in the hepatic blood flow and/or due to hepatocellular damage or any compromises of liver function will result in increases of serum levels of total bile acids. Serum or plasma TBA levels is a sensitive indicator of liver function in all species, reflecting both hepatic synthesis, secretion, and re-absorptive functions. Therefore, testing for serum TBA will help to detect liver functional changes before the formation of more advanced clinical signs of illness such as icterus. This early sensitivity is very important in clinical diagnosis because it allows for the possibility of treatment before extensive and irreversible damage is done. Studies in humans with various liver diseases showed that serum TBA can be used to assess hepatic dysfunction with valuable information that is not provided by conventional tests on serum levels of liver enzymes such as ALT and AST. It is important to distinguish between the information provided by liver enzymes (ALT, AST) and TBA. ALT and AST are enzymes released from damaged liver cells and therefore are indicators of hepatocellular integrity. TBA is an indicator of liver function. However, the test will not provide a definitive diagnostics of the primary problem, merely on early confirmation that there is a hepatobiliary deficiency. Therefore, once a patient becomes jaundiced, the benefits of TBA testing decline unless used to monitor response to treatments.

## Prevalence of Liver Disease and Screening Tests

The World Health Organization (WHO) estimates there are 12 million acute and chronic liver failure patients worldwide. The prevalence of liver disease is particularly high in developing countries, especially in Asia.

Liver disease is a major medical problem in China, where it results in more than 400,000 deaths per year. Hepatitis is the third most prevalent disease in China, and 20 million people have active viral liver disease. Official estimates suggest that China's yearly medical expenses for liver disease infections are more than \$12 billion.

In the United States, the National Center for Health Statistics and the American Liver Foundation estimates that:

- *Over 26,000 people die each year from chronic liver Cirrhosis, a chronic liver disease that is the seventh leading disease-related cause of death in the US.*
- *Approximately 3.5 million people in the US are chronically infected with the hepatitis C virus.*
- *Between 8,000 and 10,000 people die of hepatitis C annually in the US. By the year 2010, the number of deaths from hepatitis C is expected to rise to 38,000 each year.*
- *There are approximately 22,000 pregnant women who are carriers of hepatitis B each year in the US.*
- *Each year, 400,000 to 500,000 surgeries to remove the gallbladder are performed in the US.*

Early detection of liver disease and liver functionality can help patients get effective therapeutic treatment, prevent disease progress, and save lives. Liver health screening test panels normally include the following tests:

- *Liver enzymes: ALT, AST, GGT, AFU, ADA, ChE.*
- *Liver tumor marker: AFP.*
- *Liver function markers: Bilirubin (total and direct), TBA*

Among these tests, TBA offers the highest sensitivity for early stage of liver dysfunction. This test as part of the liver test panel has been widely performed in China and other Asian countries for early detection of liver diseases.

## Methods for Serum Total Bile Acids Test

Several assays have been used to determine both total or individual bile acids in biological fluids. The methods that have been used specifically to analyze serum TBA are gas-liquid chromatography (GLC), High Performance Liquid Chromatography (HPLC), enzymatic assays and enzyme cycling assays. GLC and HPLC methods are not commonly used in clinical laboratories where automated clinical chemistry analyzers are used for most of chemistry tests including TBA testing. The enzymatic assay (so called third generation TBA assay) is now mainly used in small laboratories where manual operations are allowed as the reagents of the 3rd generation TBA test are in lyophilized powder form, and manual reconstitution steps are needed before use. At present, the most widely used TBA test in clinical laboratories is the enzyme cycling method (also called the 5th generation TBA assay). That is a liquid-stable assay and ready to use for all types of automated chemistry analyzers.

### 1. Enzymatic method (3rd generation):

The enzymatic TBA assay method, as depicted in Figure 4, uses an enzyme, 3- $\alpha$ -hydroxysteroid dehydrogenase (3 $\alpha$ -HSD), to catalyze the oxidation reaction converting 3- $\alpha$ -hydroxyl group of all bile acids to 3-keto group with concomitant formation of a co-enzyme NADH from NAD<sup>+</sup>. The NADH formed is further reacted with nitrotetrazolium blue (NBT) to form a formazan dye in the presence of diaphorase enzyme. The dye formation is monitored by measuring the absorbance at 540 nm, which is directly proportional to the bile acids concentration in the serum sample.

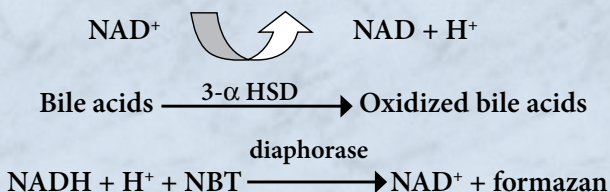


Figure 4. Assay Principle

## 2. Enzyme Cycling Method (5th generation):

The enzyme cycling assay is depicted in Figure 5 and is a method that allows for signal amplification through cycled regeneration reactions. In the enzyme cycling based TBA assay, serum bile acids molecules are repeatedly oxidized and reduced by the enzyme 3- $\alpha$ -hydroxysteroid dehydrogenase (3- $\alpha$ -HSD) with a concomitant accumulation of reduced co-enzyme thio-NADH that is detected at a specific wavelength (405 nm).

As shown in the reaction scheme below, in the forward reaction, the enzyme catalyzes the oxidation reaction in the presence of co-enzyme thio-NAD<sup>+</sup> to form oxidized bile acids and reduced co-enzyme thio-NADH. On the other hand, in the reverse reaction, the enzyme catalyzes the reduction reaction in the presence of excess co-enzyme NADH to convert oxidized bile acids back to bile acids which are then ready for the next round of forward oxidization reaction. The innovative use of this paired co-enzyme and co-enzyme analog enables a significant signal amplification, and therefore leads to a much higher detection sensitivity of the assay. The rate of thio-NADH formation is detected at 405 nm, and is proportional to the amount of TBA in the sample. The enzyme cycling TBA assay offers analytical performance far beyond the capabilities of conventional bile acids test methods.

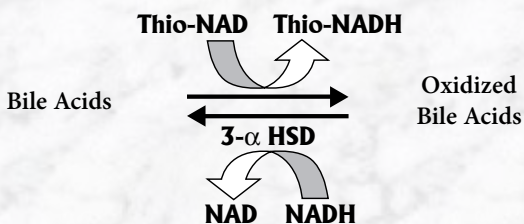


Figure 5. Assay Principle

## TBA Assay Methods Comparison

Table 2 lists the advantages and disadvantages of enzymatic and enzyme cycling methods for TBA assay. The major advantages of the enzyme cycling assays over the conventional enzymatic assays are:

- *Liquid stable, ready to use*
- *High detection sensitivity*
- *Less interference from lipemic and hemolytic samples*
- *Less sample volume needed*
- *No instrumentation contamination by formazan dye*

	Enzymatic (NBT)	Enzyme Cycling (Thio-NAD)
Reagent format	Lyophilized powder	Liquid Stable
Detection wavelength	540 nm	405 nm
Sample volume	20 uL	3-5 uL
Interference by lipemic samples	Yes	No
Interference by hemolytic samples	Yes	No
Instrument contamination	Yes	No

**Table 2. TBA assay methods comparison**

Figures 6 and 7 show the effects of triglyceride and hemoglobin on TBA assays of enzymatic colorimetric method (NBT method) and enzyme cycling method (thio-NAD method). As seen from these figures, the enzyme cycling based TBA assay method has significantly less lipemic and hemolytic interferences.

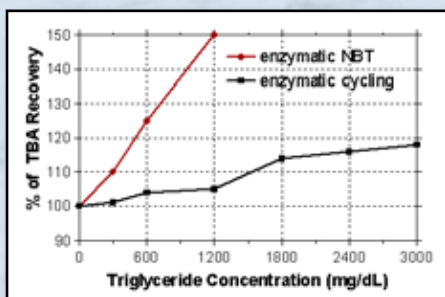


Figure 6.

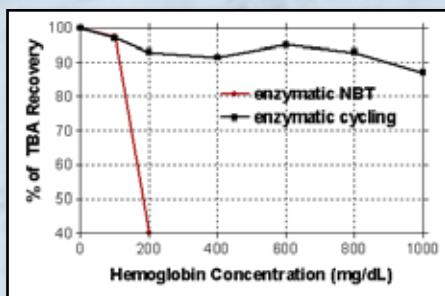


Figure 7.

## Serum Total Bile Acids and Diagnosis of Liver Diseases

Fasting serum TBA determination can be used clinically in the diagnosis and prognosis of liver disease in conjunction with standard liver function tests. Because of the increased sensitivity of TBA determination as compared to standard liver function tests, TBA testing offers significant additional diagnostic information concerning liver function, especially in minor hepatic derangements. It is of particular benefit in the determination of hepatic dysfunction as a result of chemical and environmental injury.

Liver injury as a result of occupational or environmental exposure to a wide variety of chemical substances can be determined to a much finer degree by TBA than by standard liver enzymes, especially when the liver has been only slightly damaged. Studies showed that 73% of patients exposed to harmful organic solvents had elevated serum TBA levels, whereas increased levels of gamma-glutamyl transpeptidase ( $\gamma$ -GT), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin were only 8, 3, 2 and 1%, respectively. Clinical studies have found that

standard liver function tests are not sensitive enough to determine hepatic dysfunction caused by organic solvent exposure, whereas serum TBA testing has a much greater specificity and sensitivity in the diagnosis of liver disease induced by chemical and environmental exposure and in diagnosis of low levels of hepatic dysfunction.

Other indications for serum TBA testing include patients presenting with generalized pruritis and pregnant women experiencing nausea and vomiting during pregnancy. Both of these conditions can be a result of impaired hepatic function, yet standard liver function tests are usually not sensitive enough to be of value. In contrast, serum TBA determination has shown a significant correlation between hepatic dysfunction and both nausea and vomiting during pregnancy and generalized pruritis.

Serum TBA testing also offers useful prognostic information in cases of cirrhosis. In one large study, serum TBA concentration correlated more closely with mortality than the commonly used clinical and laboratory parameters such as the Number Connection Test, acites, albumin, pseudocholinesterase, bilirubin, prothrombin time, and nutritional state.

Serum TBA testing is generally not suitable for differentiating between the various types of liver diseases.

**Table 3. Conditions with elevated fasting serum total bile acids levels**

- Anicteric liver disease
- Alcoholic liver disease
- Biliary atresia
- Chemical-induced liver injury
- Cirrhosis
- Cholestasis
- Cystic fibrosis
- Drug-induced liver injury
- Generalized pruritis
- Hepatoma, primary
- Nausea and vomiting of pregnancy
- Neonatal hepatitis syndrome
- Protracted diarrhea of infancy
- Reye's syndrome
- Viral hepatitis

# Normal Range of Fasting Total Bile Acids in Human Serum

Numerous studies have shown that the normal range of fasting total bile acids levels in human serum is 2-10  $\mu\text{mole/L}$ .

Postprandial serum TBA levels are generally higher than fasting serum TBA levels. It has recently been proposed that measurement of both fasting and postprandial serum TBA levels can provide additional value in differential diagnosis of chronic liver dysfunctions, (as seen in Table 4).

	Fasting (12 hr) <10 $\mu\text{mol/L}$	Post-prandial (2 hr) <20 $\mu\text{mol/L}$	Comment
Bile Duct Obstruction	>180 $\mu\text{mol/L}$	>180 $\mu\text{mol/L}$	Highly elevated with essentially no difference between fasting and post-prandial levels
Intrahepatic Cholestasis	~ 100 $\mu\text{mol/L}$	~ 120 $\mu\text{mol/L}$	Serum levels lower than blockage outside the liver
Portosystemic Shunt	<10 $\mu\text{mol/L}$	>180 $\mu\text{mol/L}$	Direct communication between hepatic-portal and general circulation without the liver having the opportunity to filter
Inadequate fast decreased GI motility or spontaneous gall bladder contraction	25-50 $\mu\text{mol/L}$	<20 $\mu\text{mol/L}$	Fasting level higher than post-prandial levels
Prolonged fast, intestinal malabsorption, increased GI motility normal variation	10 $\mu\text{mol/L}$	10 $\mu\text{mol/L}$	

Table 4. Fasting vs postprandial levels of serum TBA in diagnosis of liver diseases

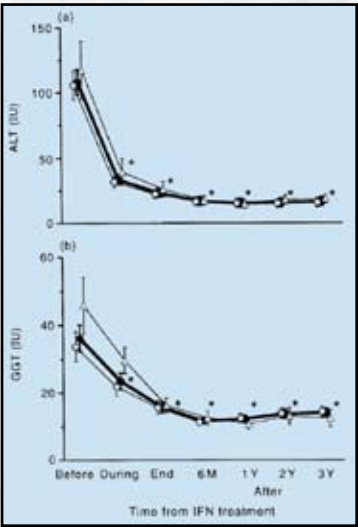
## Serum Total Bile Acids Level As A Sensitive Prognostic Test for HCV Patients Treated with Interferon

Toshihide Sima et al.(J. Gastroenterol. hepatol. 15: 294-299, 2000) recently reported that serum TBA levels is a sensitive indicator of hepatic histological improvement in chronic hepatitis C patients responding to interferon treatment. A decrease in serum TBA levels reflects histological improvement in the liver more precisely than changes of other liver function test values following Interferon therapy (IFN).

IFN has been widely used for treatment of chronic hepatitis C virus (HCV) infection since the late 1980s and is still the most approved treatment for chronic hepatitis C. Approximately one-third of IFN-treated patients with chronic hepatitis C show long-term favorable responses, including the eradication of HCV, normalization of liver function test values, and improvement in liver histology. TBA has been revealed to be more sensitive than other conventional live function tests (ALT, AST, GGT total bilirubin, albumin, and colinesterase (ChE)) in detecting liver dysfunction, and the monitoring of TBA has been reported to be useful for determining the clinical course of chronic liver diseases. For example, in patients with whom compensated liver cirrhosis was progressing into the decompensated form, TBA levels increased before changes in other liver function test values occurred.

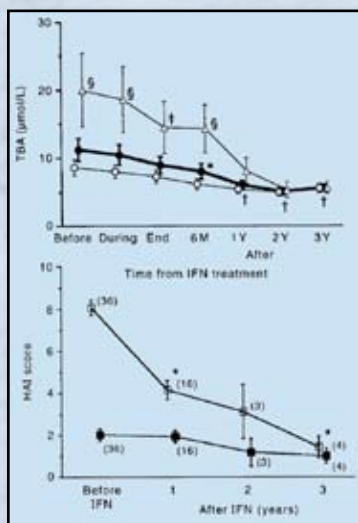
During the course of IFN treatment for HCV patients, various liver function tests including serum TBA were performed and the sensitivities of these tests in responding to interferon treatment were compared (See Figures 6a to 6d).

Data in Figure 8 depict the changes of test values before and after IFN treatment separated by groups of the all responders (●), responders with mild chronic active hepatitis (○), and severe chronic active hepatitis (Δ).



As seen in Figure 8a, and 8b, there were no differentiations in values of ALT and GGT between mild and severe chronic hepatitis. In contrast, serum TBA values (Figure 8c) clearly differentiate mild chronic active hepatitis from severe chronic active hepatitis, indicating that serum TBA is a more sensitive indicator predicting the severeness of liver dysfunction.

Figure 8a and 8b.



**Figure 8c and 8d.**

More importantly, none of the conventional liver function tests (ALT, AST, GGT, albumin, bilirubin, and ChE) show any correlation with patient's liver histological improvements following IFN therapy. ALT and AST values indicate hepatocellular necrosis and GGT value reflects both cholestasis and hepatocellular injuries. These conventional liver function markers significantly decreased and normalized during the first 6 months of IFN treatment, whereas the grading scores of histological activity indices (HAI) were still elevated at the end of a 6 months treatment period as shown in Figure 8d. The HAI grading score (■) and staging scores (●) decreased gradually over the 3 year follow-up period, and its patient matched well with the serum TBA values which also gradually decreased during the 3 year follow-up period. For patients with abnormal TBA values before IFN treatment, there was a significant correlation between the histological improvement in grading scores and serum TBA levels. The TBA value more accurately reflects the overall state of the liver as compared to other liver function tests, so a slow improvement in TBA value suggests that the functional and histological recovery of the damaged liver may extend over a few years, even after the eradication of HCV. Therefore, for severe chronic active hepatitis patients who had an abnormal TBA value before IFN treatment, it is clear that the change in TBA levels is the most sensitive biochemical indicator of hepatic histological improvement after successful IFN treatment for chronic hepatitis C. Hence, prognostic testing of serum TBA provides valuable information on the effectiveness of IFN treatment and the degree of liver histological improvement during and after treatment.

# Serum Total Bile Acids Level As an Indicator for Intrahepatic Cholestasis of Pregnancy



Some women experience severe itching during late pregnancy. The most common cause of this is Cholestasis; a common liver disease that only happens during pregnancy. Cholestasis of pregnancy is a condition in which the normal flow of bile in the gallbladder is affected by the high amounts of hormones released during pregnancy. Cholestasis is more common in the last trimester of pregnancy when hormonal activity are at their peak, but usually subsides within a few days after delivery. Cholestasis of pregnancy is also referred to as intrahepatic cholestasis of pregnancy (ICP) or obstetric cholestasis.

## What causes Cholestasis of pregnancy?

Pregnancy hormones affect gallbladder function, resulting in slowing or stopping of the flow of bile. The gallbladder holds bile that is produced in the liver, which is necessary in the breakdown of fats in digestion. When the bile flow is stopped or slowed down, this causes a build up of bile acids in the liver which can spill into the bloodstream, and leads to significantly increased serum TBA levels as shown in Figure 9.

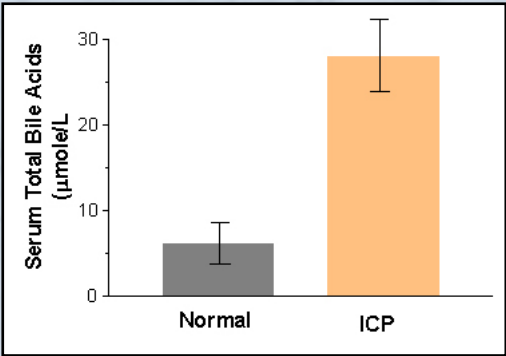


Figure 9.

### *What are the symptoms of Cholestasis of pregnancy?*

- *Itching, particularly on the hands and feet (often is the only symptom noticed)*
- *Dark-colored urine*
- *Light-colored bowel movements*
- *Fatigue or exhaustion*
- *Loss of appetite*
- *Depression*

#### *Less common symptoms include:*

- *Jaundice (yellow coloring of skin, eyes, and mucous membranes)*
- *Upper-Right Quadrant Pain*
- *Nausea*

### *Who is at risk for Cholestasis of pregnancy?*

1 to 2 pregnant women in 1000 are affected by Cholestasis in North America and European countries. It is more common in some South American countries, especially Chile and Bolivia, where up to 1 in 10 (or more) pregnant women develop this condition. In general, the following women have a higher risk of developing Cholestasis during pregnancy:

- *Women carrying multiples*
- *Women having previous liver damage*
- *Women whose mother or sisters had Cholestasis*

### *How is Cholestasis of pregnancy diagnosed?*

A diagnosis of Cholestasis can be made by doing a complete medical history, physical examination, and blood tests that evaluate liver function, total bile acids, and bilirubin.

### *How will the baby be affected if the mother is diagnosed with Cholestasis?*

Cholestasis may increase the risks for fetal distress, preterm birth, or stillbirth. A developing baby relies on the mother's liver to remove bile acids from the blood, therefore the elevated levels of maternal bile cause stress on the baby's liver. Women with Cholestasis should be monitored closely and serious consideration should be given to inducing labor once the baby's lungs have reached maturity.

### *What is the treatment for Cholestasis of Pregnancy?*

The treatment goals for Cholestasis of Pregnancy are to relieve itching. Some treatment options include:

- *Topical anti-itch medications or medication with corticosteroids*
- *Medication to decrease the concentration of bile acids such as ursodeoxycholic acid*
- *Cold baths and ice water slows down the flow of blood in the body by decreasing it's temperature.*
- *Dexamethasone is a steroid that increases the maturity of the baby's lungs.*
- *Vitamin K supplements administered to the mother before delivery and again once the baby is born to prevent intracranial hemorrhaging.*
- *Regular blood tests monitoring both serum TBA levels and liver functions.*

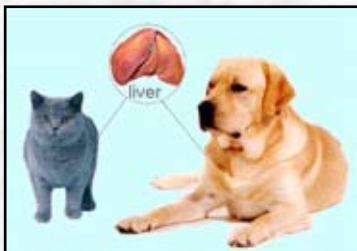
Treatment for Cholestasis of Pregnancy needs to be determined by your physician who will take the following criteria into consideration:

- *Your pregnancy, overall health, and medical history*
- *The extent of the disease*
- *Your tolerance of specific medications, procedures, or therapies*
- *Expectations for the course of the disease*
- *Your opinion or preference*

# Serum Total Bile Acids Test in Veterinary Clinics

Determination of serum TBA is a common diagnostic test for animal hepatic functions in veterinary laboratories. While there are several different liver-function tests available, serum TBA test is the most sensitive,

the easiest to perform and the most liver-specific. TBA testing is also useful in avian medicine because elevated liver enzyme activity in birds, such as increased AST, does not always correlate with the presence of liver disease.



Testing for serum TBA bile detects liver changes before the development of clinical signs such as icterus. This early sensitivity is extremely important because it allows for the possibility of treatment before the development of extensive and irreversible liver damage of animals.

## *Clinical Interpretation*

### *Increased values:*

In the presence of impaired hepatic anion transport, which can be induced by a variety of hepatic diseases, serum TBA levels can be expected to rise markedly from 100  $\mu\text{mole/L}$  to over 350  $\mu\text{mole/L}$  in severe cases. This test has generally replaced the BSP clearance test as the indicator of choice in hepatic anion transport and has been used successfully in many species of animals and birds.

Because of the enterohepatic circulation, an evaluation of gut function must be made before interpreting TBA results, as lower than expected concentrations could occur due to impaired re-absorption. The test should compliment standard tests for evidence of liver function/disease rather than act as a replacement. In monogastric animals, the pre- and post- prandial measurement of TBA is a very sensitive measure of hepatic biliary disease due to the normal increase in secretion with eating and subsequent re-absorption into the blood stream. In healthy animals, serum TBA concentrations return to normal baseline levels within two hours after eating.

Serum TBA tests have replaced the use of plasma ammonia tests in the detection of hepato-portal shunts.

***Low values:***

Extremely low values of serum TBA may be seen with intestinal blockage by foreign bodies and stasis.

***Reference Ranges***

The following series of reference values for serum TBA are currently in use and can be used as an aid to interpretation. It should be noted that these reference values were determined using enzymatic TBA assay method, and should not be compared with the values quoted in literature using radio-immuno assay.

Species	Range ( $\mu$ mole/L)
Sheep	0-50
Cattle	0-50
Goat	0-50
Pig	0-50
Horse	0-15
Dog-fasting	0-30
Dog-postprandial	0-50
Cat-fasting	0-10
Cat-postprandial	0-30
Birds	0-100

**Table 5.** Reference ranges serum TBA in animals

***Sample handling:***

To obtain the best results, there are some basics to consider when performing this assay:

A 12-hour fast must be undertaken prior to the first (pre-prandial) sample.

It is very important to perform a postprandial sample, as well as a fasting sample, or the diagnosis may be missed.

The amount and type of food used with this assay are im-

portant. While the amount of food is not known for sure, general recommendations are to feed at least 2 teaspoons of food to animals that weigh less than 5 kg, and approximately 1/4 can of food for larger animals. You don't want to overfeed because lipemia can adversely affect the bile acids results, and you should avoid foods with low-fat and low-protein concentrations.

Hemolysis can adversely affect your test results. However, when enzyme cycling based TBA test is used, lipemia and hemolytic samples are more tolerated.

A serum sample is preferred for the TBA test. However, when serum is not available, a heparinized plasma sample can also be used, but the recovery of TBA is only 90% of serum TBA.

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please contact us at:*

**DIAZYME LABORATORIES**

12889 Gregg Court  
Poway, California 92064

Tel: 001-858-455-4768

Fax: 001-858-455-3701

